

Basic protocol for antibody and antigen incubations: FAST slides printed with anti-peptide tag capture antibodies

After printing anti-peptide tag capture antibodies onto FAST slides, the slides were allowed to dry as described. Slides were then blocked with BBSA-T, for 30 min to 1 [hr]h, at 37°C in a shaking incubator (37°C incubations).

Purified scFvs, containing peptide tags, were then diluted to various concentrations (typically between 0.1 and 100 µg/ml) in BBSA-T. Slides containing anti-peptide tag capture antibodies were then incubated with this antigen solution for 1 [hr]h at 37°C. Slides were then washed three times with PBS-T, 3-5 min per wash, at ambient temperature.

Slides containing anti-peptide tag capture antibodies and bound scFvs were then incubated with biotinylated human fibronectin or biotinylated human glycophorin (as antigens) diluted to various concentrations (typically 1-10 µg/ml) in BBSA-T, for 1 [hr]h at 37°C. Slides were then washed with PBS-T as described above.

IN THE ABSTRACT:

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ABSTRACT

Provided herein are addressable collections of anti-tag capture agents, such as antibodies, that are used as tools for sorting proteins containing polypeptide tags for which the capture agents are specific. Also provided are methods of nested sorting using the collections. The methods [includes]include the steps of creating tagged collections of molecules by introducing a set of nucleic acid molecules that encode unique preselected polypeptides to create a library of tagged molecules; either before or after introducing the tags, dividing the library into N divisions; translating each division and reacting each with one of N capture agent collections, identifying the capture agents bound to the polypeptide tags linked to molecules of interest, and thereby identifying the one of the divided collections that contains the molecules of interest. The method